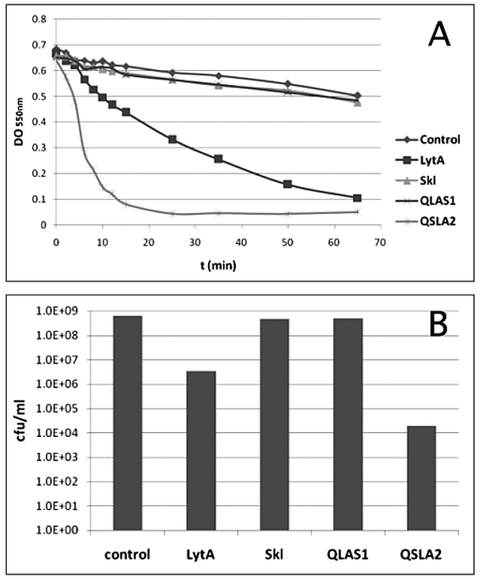

Fig. 1. (A) Antimicrobial activity of Skl, LytA, and the chimeras against strain R6 of S. pneumoniae measured by following the decrease in OD550nm. (B) Viable cells after 60 min incubation. Measurements performed at 37ºC, in PBS, pH 6.8, and 0.25 µg/ml of enzyme.

4. Conclusions

Systematic characterization of the Skl endolysin in comparison with the LytA autolysin has allowed to create a chimeric lysin, QSLA2, whose killing efficiency on *S. pneumoniae* exceeded to that of LytA.

Choline binding affinity and choline-promoted dimerization seems to be essential for the antipneumococcal activity of lysins with the same kind of CBM than LytA. Their alteration can be used as a tool to improve or modulate lysin activities.

The differences found in the antimicrobial efficiencies of LytA and QSLA2 denote the influence of the CM and the overall modular architecture of lysins, another factor that could be used in the construction of enzybiotics with enhanced activities.

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Validation of new therapeutic targets in antibacterials throughout an academic Drug Discovery Platform

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ABSTRACT:

The relentless increase in bacterial resistance to our current armamentarium of antibiotics could shortly take Europe and other global communities into a post-antibiotic era in which many common infections would be unable to be effectively treated. To avert this danger requires a fundamental change in antibacterial drug discovery that will involve the identification of new targets, the development of new chemical compounds. We present the MHit initiative, a Drug Discovery Platform that provides the framework for blending the academia innovative approaches together with their translation in real practical solutions, where the industry plays a fundamental role.

Keywords: Drug Discovery, target validation, *Streptococcus pneumoniae*.

1. Introduction

The so-called "antibiotic era of drug discovery" (1920s-1960s) witnessed the appearance of a number of molecular classes that constitute the basis of most of the antimicrobials in use today. However, discovery of fundamentally new classes of antibiotics came to an almost complete halt after the mid 1960s [1] despite the fact that the proportion of antibiotic resistant bacteria had been increasing over this period. Antimicrobial resistance causes profound problems with regard to infections due to Gram-positive microorganisms such as *Staphylococcus aureus* and *Streptococcus pneumoniae* (pneumococcus), which are among the most problematical bacterial pathogens worldwide. It is known that bacterial pneumonia is the major cause of childhood mortality worldwide along with malnutrition [2].

2. MHit Platform

The pharmaceutical industry has enjoyed a period of immense success reaching a peak market capitalisation value and contributing real benefits to global healthcare. This notwithstanding, the industry today is facing some very real issues around its overall productivity [2,3].

Unquestionably, academia has a real atmosphere for creative and innovative science, assuming day-by-day research opportunities that are often ignored by the industry. Together with the innovative value of the academia, the universities and public research institutes are today the reference for biomedical research with totally modernized infrastructures. Accordingly, potentiating research efforts in public research centres will notably contribute to increase the number of druggable target candidates [3,4].

MHit Platform is an academical initiative aimed in the identification of unexploited new bacterial targets that could provide the basis for the design and evaluation of novel drugs. MHit owns a ChemioBank with target key metabolic steps in the biosynthesis of essential cellular building blocks of surface proteins or inhibit the function of virulence determinants necessary for bacterial pathogenesis. Small antimicrobial molecules have been identified from natural and synthetic sources and *in vivo* models for human diseases are fully operational within the consortium. From 2012, *hit-to-lead* process is guided under the most stringent regulatory basis to guarantee an efficient traslation to the productive sector.

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Phenotypic changes in mutants of the *Streptococcus pneumoniae* F1/Fo ATPase

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ABSTRACT:

The F1/F0 ATPase is the main proton pump of the aerotolerant anaerobic bacterium *Streptococcus pneumoniae* and is involved in building the proton gradient of the membrane, in the homeostasis of cations, the maintenance of the intracellular pH and influences the surface charge of the cell. We have isolated from murine blood a series of pneumococcal mutants with single nucleotide polymorphisms (SNP) in different subunits of the ATPase genes including strains with SNPs in *atpA, atpB, atpC, atpD and atpG*. Since these SNPs provided a selective advantage in the host over the challenge strain TIGR4, we performed a phenotypic analysis of these mutants. Upon the assays performed, *in vivo* NMR confirmed an unaltered intracellular pH, exposure to acid evidenced decreased acid survival, and zeta-potential and hydrophobicity tests revealed changes in surface charge. Phenotype microarray data for osmolites revealed that the challenge strain TIGR4 differed from reference strain D39 by decreased resistance to a series of compounds including sodium phosphate, sodium nitrate, sodium sulphate, sodium lactate, and ethylene glycol. Interestingly in four out five mutants assayed the resistance to osmolites had increased from the level of their parental strain TIGR4 to that of D39. While having important evidence for the fitness selection of a single virulent strain during experimental infection, these mutants are an exceptional tool to investigate into the physiological role of the essential F1/F0 ATPase enzyme complex of *S. pneumoniae* during infection of the host.